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Original Article

INVESTIGATION OF THE LIPID AND CARBOHYDRATE CONTENTS OF *GREWIA TENAX* FORSSK. FRUITS & EVALUATION OF HEPATOPROTECTION ACTIVITY

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ABSTRACT

Objective: *Grewia tenax* Forssk. is represented in Egypt and known for its edible fruits which are nutritionally balanced and rich in iron and calcium. This work was to investigate the Lipid and Carbohydrate Contents of *Grewia tenax* Forssk. fruits and evaluation of Hepatoprotection activity.

Methods: Investigation of carbohydrate contents of *Grewia tenax* Forssk. fruits were carried out using HPLC. The lipids content of *Grewia tenax* Forssk. fruits were analyzed by GLC. Also, the hepatoprotective activity on rat hepatocytes monolayer culture was studied.

Results: The HPLC analysis of free sugars of *Grewia tenax* Forssk. fruits revealed the presence of stachyose, sucrose, glucose and fructose. While the HPLC analysis of the polysaccharide hydrolysate from *Grewia tenax* Forssk. fruits showed the presence of glucuronic acid, glucose, galactose and arabinose.

GLC of the unsaponifiable matter of hexane extract of *Grewia tenax* Forssk. fruits revealed the presence of a mixture of a series of n-alkanes (63%) and a series of sterols (9.9%). A series of hydrocarbons ranging from C_{10}-C_{26} in addition to cholesterol, campasterol, stigmasterol and β-Sitosterol and six fatty acids among which Linoleic acid ($C_{18:2}$) was the major one (55.5 %) was identified. Also, the hepatoprotective activity study of the extracts of *Grewia tenax* Forssk. fruits (80% MeOH extract of defatted powder) and water extract indicated that they exhibited hepatoprotection at (25 µg/ml and 50 µg/ml) and (12.5 µg/ml to 100 µg/ml); respectively.

Conclusion: Our study was the first record of carbohydrate and lipid fraction contents of *Grewia tenax* Forssk. fruits. From the obtained results, the highly nutritive and medicinal values of *Grewia tenax* Forssk. fruits should be considered. Furthermore, and due to the high hepatoprotection effects of the plant extracts, further investigation and studies should be done to isolate the bioactive compounds.

Keywords: *Grewia tenax* Forssk., Carbohydrates, Unsaponifiable fraction, Fatty acid methylester, Hepatoprotection activity.

INTRODUCTION

The genus *Grewia*, belonging to the family Teliaceae, composed of about 150 species forming an important economical complex, distributed in tropical and subtropical regions in Asia, Australia and Africa [1]. The genus *Grewia* is represented in Egypt by three species, *Grewia villosa* Willd, *Grewia tembensis* Fresen. and *Grewia tenax* Forssk. [2]. The species is known for its edible fruits, which are nutritionally balanced and rich in iron and calcium. The drupes also contain amino acids, mineral elements (K, Ca, Mn, Fe, Cu and Zn), tannin and pectic substances [3, 4]. Fruits and other parts of *Grewia tenax* Forssk. contribute significantly to the food and energy needs of rural populations in multiple ways. Its leaves and twigs are palatable fodder for livestock. The fruit, known locally in Sudan as "Gudaim", is a rich source of carbohydrates, protein, vitamins and minerals and constitutes important contributors to improving the nutritional contents of rural and urban people in Sudan [5, 6]. Moreover, the fruits are made into a fermented drink in Sudan and Southern Africa [7]. Due to containing large amounts of iron [8], *Grewia tenax* Forssk. fruit is used for treatment of anemia and malaria [9].

Phytochemically, the genus *Grewia* has been found to possess mainly triterpenoids, fatty component, flavonoids, steroids, saponins and tannins [10]. Chemical investigation of the stem bark of *Grewia tenax* Forssk. revealed the presence of triacontan-1-ol, β-amyrin, β-sitosterol, lupenone, erythrodiol, α-amyrin, and betulin [11]. Biologically, it was reported that high doses of the aqueous extract of *Grewia tenax* Forssk. fruits reduced significantly iron uptake suggesting a probable toxic effect of this extract. Histological studies confirmed the presence of cytotoxic signs [12]. The methanolic extract of *Grewia tenax* Forssk. showed good activity against *Pseudomonas aeruginosa* [13]. In this study, the carbohydrate and lipids content were investigated for the first time, in addition to the hepatoprotection activity of the prepared extracts from *Grewia tenax* Forssk. fruits

MATERIALS AND METHODS

Plant material

Fruits of *Grewia tenax* Forssk. were purchased from the local herbal market in El-Khartoum (Sudan) and were identified by Professor Kamal Zayed, Professor of Ecology, Faculty of Science, Cairo University. The fruits were sorted, washed with tap water, dried and then minced. The minced fruits were kept in the refrigerator at 4 °C before analysis.

Investigation of carbohydrate content

Five g of dried minced fruits of *Grewia tenax* Forssk. was extracted with distilled water till exhaustion. The extracts were combined and concentrated under reduced pressure at 70 °C. The concentrated solution was precipitated with the addition of ethanol and the precipitate was washed in turn with ethanol, diethylether and acetone to give polysaccharide (0.97 g). Aqueous layer (containing free sugars) was evaporated under reduced pressure to give (0.69 g) of free sugars [**14**].

Acid hydrolysis of the polysaccharides

About 200 mg of the obtained polysaccharides was heated in 2 ml of 0.5 M sulphuric acid in a sealed tube for 20 hrs on a boiling-water bath. At the end of the hydrolysis, a brown flocculent precipitate was noticed, which was filtered off and the filtrate was freed of (SO_4^{-2}) by precipitation with barium carbonate and filtered. The filtrate was evaporated under reduced pressure yielding 40 mg of polysaccharide hydrolysate.

HPLC analysis of free sugars and polysaccharide hydrolysate

Five mg of free sugars and polysaccharide hydrolysate was dissolved in one ml distilled water and analyzed with Shimadzu HPLC model (SCL-10A) equipped with liquid chromatography (LC-10D), degasser (DGU-14A), refractive index detector (RID-10A), column oven (CTO-10 AC) and Shodex sugar column (SC 1011 No. H 706087). The mobile phase was distilled water with the flow rate two ml/min. The sugars were identified in comparison with authentic reference sugars.

Investigation of lipid constituents [**15**]

Preparation of the lipoidal matter

100 g of the minced fruits of *Grewia tenax* Forssk. were extracted with hexane. The hexane extract was evaporated under reduced pressure at 40 °C yielding 1.08 g oil.

Saponification of total lipid fraction

The obtained oil was saponified by refluxing with 25 ml N/2 alcoholic KOH for 8 hrs. The alcoholic solution was concentrated to about 10 ml and diluted with cold distilled water. The unsaponifiable matter was extracted by shaking with successive portions of diethyl ether (3 x 10 ml). The combined ethereal extract was washed with distilled water, dehydrated over anhydrous sodium sulphate and evaporated under reduced pressure at 40 °C till dryness.

Preparation of total fatty acids

The residual aqueous solution left after extraction of unsaponifiable matter was rendered acidic (pH=2) with sulphuric acid. The liberated fatty acids were thoroughly extracted several times with diethyl ether. The combined ethereal extract was washed with distilled water till free of acidity and dehydrated over anhydrous sodium sulphate. The solvent was evaporated under reduced pressure at 40 °C till dryness.

Preparation of fatty acids as methyl esters

Total fatty acids fraction was dissolved in 10 ml dry methanol containing 4.5% HCl and refluxed on a boiling water bath for 3 hours. The reaction mixture was diluted with distilled water and extracted with successive portions of diethyl ether (3 x 10 ml). The combined ether extract was washed with distilled water till free of acidity, dried over sodium sulphate, filtered, and the solvent was evaporated under reduced pressure at 40 °C.

GLC analysis of the unsaponifiable matter and fatty acid methyl esters

The unsaponifiable matter and fatty acid methyl esters investigation were carried out using Agilent Technologies 6890N series GC system, Capillary Column (Zb-5), (length 30 m, diameter 530 µm and film thickness: 0.5 µm), Oven: Initial temperature: 80 °C (for 1.00 min), final temperature (250 °C), rate (8 °C/min),, inlet temperature: 250 °C (split 15:1), flow rate: 5 ml/min, detector: FID (Flame Ionization Detector), at temperature 300 °C, carrier gas: H_2, N_2 and air flow at rate: 30 ml/min.

Rat hepatocytes monolayer culture

Isolation and preparation of rat hepatocytes monolayer culture [16]

A primary culture of rat hepatocytes was prepared according to the Seglenm method [17], which was modified by Kiso [18], using a Wistar male rat (250–300 g).

The rat was obtained from the animal house of the NRC (National Research Center, Cairo). Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals [19].

LC_{50} determination on rat hepatocytes monolayer culture [16]

After 22–24 hours, the rat hepatocyte monolayer was washed twice with phosphate buffer saline (PBS). In order to determine lC_{50}, different concentrations were prepared for each sample (250-1000 µg/ml). After 2 hours of cells incubation with the extract, cell viability was determined using the MTT assay. The assay was performed according to the method of Mosmann [20] modified by Carmichael [21].

Absorbance of formasan crystals produced by viable cells was read at 540 and 630 nm dual wavelength using the Automatic Kinetic Microplate Reader (Labsystems Multiskan RC reader). Each experiment was repeated three times, and the mean absorption of each concentration was calculated. A graph plotted with x-axis showing the different concentrations of the extract used and the y-axis showing the absorbance percentage of viable cells. The lC_{50} was graphically determined from the concentration that yielded an absorption coinciding with the 50% of cells that received no extract.

Evaluation of hepatoprotection activity [16]

The primary rat hepatocyte monolayer was prepared as in Section described before. Different concentrations were prepared from *Grewia tenax* Forssk. (12.5–100 µg/ml) using the serial dilutions technique by dissolving in DMSO (1% maximum concentration). For each concentration, three replicates were carried out; in addition to positive control, that was 50µg/ml Silymarin.

The plate was incubated for 2 hours at 37°C and 5% CO_2, and then washed twice with PBS. A 200 µl of 25 mM paracetamol was added to each well. After an hour of cells incubation with the paracetamol, cell viability was determined using the MTT assay. The concentration of the extract that was able to protect the cells from the hepatotoxic effect of paracetamol was considered hepatoprotective.

RESULTS AND DISCUSSION

Investigation of carbohydrate content

The HPLC analysis of free sugars of *Grewia tenax* Forssk. fruits revealed the presence of stachyose, sucrose, glucose and fructose. While the HPLC analysis of the polysaccharide hydrolysate from *Grewia tenax* Forssk. fruits revealed the presence of glucuronic acid, glucose, galactose and arabinose.

Investigation of lipid constituents

The results of GLC of the unsaponifiable matter of hexane extract of *Grewia tenax* Forssk. fruits indicated that it consists mainly of a mixture of a series of n-alkanes (63%) and a series of sterols (9.9%), the total unidentified compounds being (27.7%) (table 1).

GLC analysis of the fatty acids from *Grewia tenax* Forssk. fruits as methyl esters revealed the presence of 6 fatty acids: myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, (0.2%, 13.6%, 6.9%, 18.5%, 55.5%, and 2.4%, respectively) (table 2).

Linoleic acid was found to be the main fatty acid (55.5 %). The oil from the fruit of the plant is rich in unsaturated fatty acids (76.4%) which could be of nutritive and medicinal value.

LC_{50} determination on rat hepatocytes monolayer culture

The viability assay was applied with a broad range of concentrations of the studied extracts (from 125-1000 µg/ml) on monolayer of rat hepatocytes. It revealed that the 80% methanolic extract of defatted powder of *Grewia tenax* Forssk. fruits had lC_{50} effect on monolayers of rat hepatocytes at the concentration 1000 µg/ml, and water extract had no lC_{50} from 125-1000 µg/ml (fig. 1).

Evaluation of hepatoprotection activity

The hepatoprotective activity results of *Grewia tenax* Forssk. fruits extract (80% MeOH extract of defatted powder) and water extract indicate that they exhibited hepatoprotection at (25 µg/ml and 50 µg/ml) and (12.5 µg/ml to 100 µg/ml) respectively (fig. 2).

The study of the carbohydrate content of *Grewia tenax* Forssk. fruits revealed the presence of stachyose, sucrose, glucose and fructose as free sugars. While the HPLC analysis of the polysaccharide hydrolysate revealed the presence of glucuronic acid, glucose, galactose and arabinose.

Fractionation of the constituents of the hexane extract of *Grewia tenax* Forssk. fruits was carried out and the components of various fractions were identified by GLC. The unsaponifiable fraction was found to contain a series of hydrocarbons ranging from C_{10}-C_{26} in addition to cholesterol, campesterol, stigmasterol and β-Sitosterol in which C_{23} (n-tricosane, 12.7 %) was the main component. Also, the study of the fatty acids by GLC analysis of their methyl esters revealed the presence of 6 fatty acids (Myristic acid ($C_{14:0}$), Palmitic acid ($C_{16:0}$), Stearic acid ($C_{18:0}$), Oleic acid ($C_{18:1}$), Linoleic acid ($C_{18:2}$) and Linolenic acid ($C_{18:3}$) in which Linoleic acid ($C_{18:2}$, 55.5%) represented the main acid. One of the important results was that the oil from the plant fruits is rich in unsaturated fatty acids (76.4%) which could be of nutritive and medicinal value.

On reviewing the literature, it was found that this is the first record of the carbohydrate contents and lipid fractions from *Grewia tenax* Forssk. fruits.

Table 1: GLC analysis of USM of *Grewia tenax* Forssk. fruit

S. No.	Compound (Comparable with)	Relative retention time* (RRt)	Relative area percentage
1	C_{10}	0.25	0.9
2	C_{11} n-decane (n-undecane)	0.43	0.1
3	C_{12} n-dodecane	0.62	0.2
4	C_{13} n-tridecane	0.73	0.4
5	C_{14} n-tetradecane	0.79	0.4
6	C_{15} n-pentadecane	0.91	0.7
7	C_{16} n-hexadecane	1	1.3
8	C_{17} n-heptadecane	1.07	1.7
9	C_{18} n-octadecane	1.17	2.2
10	C_{19} n-nonadecane	1.31	2.9
11	C_{20} n-cosane	1.36	11
12	C_{21} n-eicosane	1.42	7.1
13	C_{22} n-docosane	1.51	5.2
14	C_{23} n-tricosane	1.64	12.7
15	C_{24} n-tetracosane	1.71	5.2
16	C_{25} n-pentacosane	1.83	5.9
17	C_{26} n-hexacosane	2.01	5.3
18	Cholesterol	2.10	2
19	Campesterol	2.18	4.2
20	Stigmaterol	2.36	1
21	β-Sitosterol	2.49	2.8
	Total identified hydrocarbons		63.2
	Total identified sterols		10.0
	Total unidentified compounds		26.8

*RR_t = Relative to hexadecane retention time = 16.09 min.

Table 2: GLC analysis of fatty acids from *Grewia tenax* Forssk. fruits as methyl esters

S. No.	Compound (Comparable with)	Relative retention time* (RR_t)	Relative area percentage
1	Myristic acid ($C_{14:0}$)	0.7	0.2
2	Palmitic acid ($C_{16:0}$)	0.83	13.6
3	Stearic acid ($C_{18:0}$)	0.97	6.9
4	Oleic acid ($C_{18:1}$)	1	18.5
5	Linoleic acid ($C_{18:2}$)	1.05	55.5
6	Linolenic acid ($C_{18:3}$)	1.12	2.4
	Total identified fatty acids		97.1
	Total identified saturated fatty acids		20.7
	Total identified unsaturated fatty acids		76.4
	Total unidentified compounds		2.9

*RR_t = Relative to Oleic acid retention time = 22.35 min.

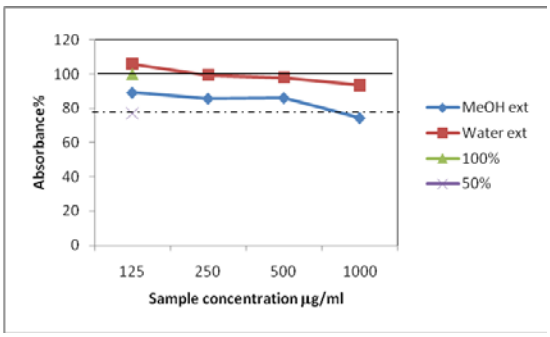

Fig. 1: Viability of monolayer of rat hepatocytes after two hours treatment with different concentrations of the extracts using MTT colorimetric assay

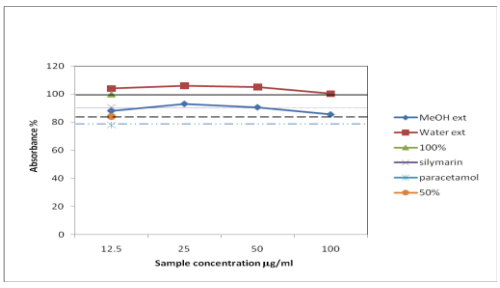

Fig. 2: Viability of monolayer of rat hepatocyte after two hours treatment with different concentrations of the extracts followed by treatment with 20 mM Paracetamol for an hour in comparison with 50 µg Silymarin as control using MTT colorimetric assay

From the hepatotoxicity and hepatoprotection results we can conclude that the tested extracts (80% MeOH extract of defatted powder and water extract) have a safety margin in all tested concentrations except for the 1000 µg/ml methanolic extract of defatted powder of *Grewia tenax* Forssk. fruits. Both extracts showed a hepatoprotection activity at certain concentrations. These results were found to be in agreement with that obtained for the antioxidant activity reported for *Grewia tenax* Forssk. fruits as reported by Saleh *et al.*, (22).

CONCLUSION

Our study was the first record of carbohydrate and lipid fraction contents of *Grewia tenax* Forssk. fruits. It revealed the presence of stachyose, sucrose, glucose and fructose as free sugars and glucuronic acid, glucose, galactose and arabinose from the polysaccharide hydrolysate, beside, a mixture of a series of n-alkanes and a series of sterols and six fatty acids. Furthermore, It was found that 80% MeOH extract of defatted powder and water extract exhibited hepatoprotection activity at (25 µg/ml and 50 µg/ml) and (12.5 µg/ml to 100 µg/ml) respectively.

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CONFLICT OF INTERESTS

Declared None

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